REMARKS

Status

Claims 87-140 were pending in this Office Action. The present response does not cancel or add any claims. Accordingly, it is claims 87-140 as herein amended which are at issue.

The Office Action

In the Office Action mailed January 5, 2010, claims 87-92, 95-102, 107-118, 120-130 and 137-140 were rejected under 35 U.S.C. §103 as being unpatentable over U.S. Patent 5,428,451 of Lea taken in view of U.S. Patent 6,280,961 of Kaplan, and further in view of European Patent Application EP 1 180 675 of Hanson and the publication of Smith.

Claims 93, 103, 110-111 and 114 were rejected under 35 U.S.C. §103 as being unpatentable over Lea taken in view of Kaplan, Hanson, and U.S. Patent 5,728,527 of Singer. Claim 94 was rejected under 35 U.S.C. §103 as being unpatentable over Lea taken in view of Kaplan and Hanson, and further in view of U.S. Patent 5,726,009 of Connors. Claims 102, 104, 107 and 131-132 were rejected under 35 U.S.C. §103 as being unpatentable over Lea taken in view of Kaplan and Hanson, and further in view of U.S. Patent 5,877,161 of Riabowol. Claims 105, 107 and 135 were rejected under 35 U.S.C. §103 as being unpatentable over Lea taken in view of Kaplan and Hanson, and further in view of U.S. Patent 5,691,147 of Draetta. Claims 106, 107 and 136 were rejected under 35 U.S.C. §103 as being unpatentable over Lea taken in view of Kaplan and Hanson, and further in view of U.S. Patent 6,379,882 of Bitler. Claim 119 was rejected under 35 U.S.C. §103 as being unpatentable over Lea taken in view of Kaplan and Hanson, and further in view of U.S. Patent 6,100,535 of Mathies. Claims 102, 104-105, 107, 131 and 133 were rejected under 35 U.S.C. §103 as being unpatentable over Lea in view of Kaplan and Hanson, and further in view of U.S. Patent 5,741,646 of Sherley. Claims 102,

104-105, 107 and 134 were rejected under 35 U.S.C. §103 as being unpatentable over Lea in view of Kaplan and Hanson, and further in view of U.S. Patent 5,344,760 of Harvey.

In addition to the prior art based rejections, claims 138, 139 and 108 were rejected under 35 U.S.C. §112 for particular noted informalities.

Applicant thanks the Examiner for the Office Action and for the thorough explanation of the basis of the rejections.

<u>The Rejections under 35 U.S.C. §112, Second Paragraph</u>

In section 4 of the Office Action, the Examiner objected to "claims 139 and 139" insofar as they were asserted to include trademark/trade names "Cy3, Cy5, and Cy5.5". Applicant presumes that the Examiner was in fact referring to claims 138 and 139 and will address the rejection in this regard.

Applicant respectfully disagrees with the Examiner's assertion that the Cy3, Cy5 and Cy5.5 designations comprise trademarks or trade names. These designations are well known in the scientific community as referring to specific cyanine dyes and are not proprietary trademarks of any party. In this regard Applicant includes herewith as Exhibit 1 an entry from Wikipedia describing various cyanine dyes and referencing the "Cy" nomenclature system and detailing its origin in a publication of Ernst et al. Applicant does note for the record that the Amersham Corporation did sell particular versions of its Cy3 and Cy5 cyanine dyes under the corresponding "Cy3 Direct" and "Cy5 Direct" trademarks. These marks were both registered; although the "Cy3 Direct" registration has lapsed. These marks refer to a particular type of Cy dye sold by Amersham. Applicant is not using the word "Direct" in the present specification or claims and is not making use of any designations which could be considered trademarks or trade names. Applicant's designations of cyanine dyes is in accord with standard scientific practice, and one

of skill in the art would readily know precisely what structures are being referred to. Reconsideration and withdrawal of this rejection is requested.

Claim 108 was rejected under 35 U.S.C. §112 as not having a sufficient antecedent basis for the recitation of "said targeting species" in line 2. Applicant thanks the Examiner for noting this informality, and per the Examiner's suggestion the claim has been rewritten to overcome this rejection.

Rejections under 35 U.S.C. §103

The claims at issue have all been rejected as being obvious in view of the prior art. The basis of all of the rejections is the combination of the Lea, Kaplan and Hanson references. Applicant's broadest claims have been rejected on the combination of these three references, and various of the narrower claims have been further rejected over the Lea, Kaplan and Hanson combination taken further with regard to other cited prior art. In view of the amendments made herein to the claims (which amendments find full support in the specification as originally filed, for example at page 21, lines 26-30 and page 22, lines 6-12), and further in view of the remarks presented herein, Applicant respectfully submits that the combination of Lea, Kaplan and Hanson does not show or suggest the principles of the present invention either taken alone or in combination with the other cited prior art.

The Present Invention

The present invention relates to a method for the detection and analysis of particles in a liquid material through the use of a reagent which comprises a labelled targeting species. The targeting species is capable of selectively binding to analyte detectable positions on the particle and the labelling agent is a compound capable of emitting, absorbing, attenuating or scattering electromagnetic radiation to result in the generation of a detectable electromagnetic signal. The

method is directed to those situations wherein the number of analyte detectable positions on the particles is very low, such as less than 1×10^6 positions per particle. As a consequence, the detectable signal produced by the reagent material is quite low. The low number of analyte detectable positions is attributed to the surface based (non-core) cell binding mechanism. Therefore, in the present invention the novel combination of optics and experimental conditions used, such as detection when the sample is at standstill, are crucial.

The invention aims to solve the technical problem of *"How to measure labelled particles with a low amount of a visual label"*, which is well correlated with the object stated in the description of the present invention on page 2, lines 13-15 stating "It is an object of the present invention to provide an alternative method for the assessment of properties related to particles based on staining of analytes which are several orders of magnitude less abundant than DNA monomers."

In contrast, any system/method relying upon fluorescent labelling of DNA (cell core labelling) in cellular material typically produces 3×10^9 detectable positions per particle; and, hence produces an intense electromagnetic signal, which can be captured from each cell in a short period of time. Accordingly, in such prior art methods, the analysis can be performed even on a moving particle sample flowing past a detection element, by hydrodynamic techniques such as flow cytometry.

Therefore, at least two factors namely: labelling mechanism resulting in small number of analyte detectable positions per particle and the detection method are significant while considering inventiveness of the present invention.

<u>The invention is non-obvious in view of Lea et al. (US 5,428,451)</u>

The Examiner states that that Lea et al. teaches a method for assessing at least one quality or quantity parameter of a particle in a liquid material.

Scope & Content of Lea

The scope and content of the cited art includes a method in which fluorescent particles are counted as they "flow" through an optical cell and past a CCD device, i.e. using hydrodynamic (as also suggested by the name itself "flow") flow cytometry technique (abstract; claim 1; col. 2, lines 19-23). In flow cytometry, the sample is transported through an interrogation point and for accurate detection of signal and data collection from each particle that passes through the interrogation point; it is important that particles or cells are passed through the beam one at a time, i.e. the flow is arranged so that there is a large separation between cells relative to their diameter to avoid any overlap between the particles. This feature of flow cytometry is implemented in the cited method as well (col. 3, line 68 – col. 4, line 2). Reliable signal detection under such hydrodynamic focusing is possible only if a large electromagnetic signal can be captured from each cell in a short period of time. This indicates that the cited art method can hence only be used for assessing parameters of particles having a large number of detectable positions (mentioned earlier in "The Present Invention" section), which is only attainable through cell core labelling.

Hydrodynamic v/s Hydrostatic

The claims of the present invention differ from the prior art, because the claimed method is not a hydrodynamic method and does not take measurements on a flowing sample. Instead, it employs an imaging system that can image cells that are at a standstill position, i.e. it is a hydrostatic method, thereby enabling hydrostatic focussing and reading of the signal from more

than one particle at any given time. This is advantageous over the hydrodynamic method of Lea because it allows for specified detected events to be revisited, after initial measurements, for a more extensive analysis. This also allows optimal use of measurement time in order to improve any signal to noise conditions and also to detect weak signals to assess particles with low detectable positions. This is certainly not the case with Lea et al.'s work, which leads a skilled person away from the present invention through its divergent hydrodynamic technique such as flow cytometry. (See, *In re Haruna*, 249 F.3d 1327, 58 USPQ2d 1517 (Fed. Cir. 2001).)

Alternative to flow cytometry

The Office Action refers to col. 4, lines 19-21 in Lea to indicate that Lea characterizes its method as being an alternative to flow cytometers. There is no dispute that the Lea method is carried out on a flowing sample and is inherently a hydrodynamic technique. In other words, Lea's method indeed is a flow cytometry method. Besides, the referred passage of Lea only suggests using a different light source, i.e. other than laser light and does not characterize the Lea method as differing from that of conventional flow cytometers. Therefore, the Office Action mistakenly interprets the statement.

Sequential v/s Simultaneous

In Lea, large separations between the cells/particles and passage of each particle through the interrogation point one-after-another results in sequential detection of only one particle at a time. In contrast, the method of the present invention requires simultaneous detection of a plurality of particles. Lea method is incapable of performing such simultaneous detection of more than one particle because there is no suggestion made in Lea about the possibility of making such a modification in the cited system of Lea. Moreover, if such modification is

incorporated in Lea's system, then the modified system would not comply with the working principle of flow cytometry.

Limitation of Flow cytometry & Flow speed control

Although, Lea et al. mentions that if the particle stream is found to be moving too fast for accurate counting, the speed may be controlled (col. 3, lines 20-30), but it should be noted that flow cytometry relies on being able to rapidly examine and distinguish between different particles in a liquid flow. In Lea and all other hydrodynamic techniques, the speed control only ensures that there is a large separation between consecutive particles, thereby avoiding any overlap. A skilled person employing flow cytometry is thus looking for a high throughput screening of particles to obtain a detectable signal. Therefore, even if the speed is reduced, no skilled person, while using flow cytometry and requiring detection of signal under hydrodynamic condition would make the sample to stand still because that would disrupt the working principle of flow cytometry as it is based on hydrodynamic detection of one particle at a time. The control of flow rate in Lea et al. is primarily and only to control the cell separation for accurate counting.

Hence, standstill sample assessment is contrary to the scope of flow cytometry technique. More specifically, the development work in flow cytometry is directed towards increasing the flow speed of the particles and scanning reliably the flowing particles individually, rather than assessing multiple particles at a time by bringing the speed down to standstill. Therefore, any skilled person using the teaching of Lea et al. would try the taught method only with particles having a high number of detectable positions because this enables detection of signals from particles under flow condition.

Speculative situation suggested in the Office Action

Because in hydrodynamic processes such as that of Lea, detection of signals from each labelled particle is performed individually and one-at-a-time, therefore, bringing the speed of the particle to zero and incorporating a thin flow path (col. 3, line 68 – col. 4, line 2), as suggested by the Office Action would at the best result in illumination of only one particle, the one which is stopped at the detection point. Consequently, the combination suggested by the Office Action will not be able to illuminate and detect a plurality of the particles in the sample simultaneously as is the case in the present invention. Moreover, as explained in the preceding paragraph, bringing the speed to zero is not in compliance with the teachings of Lea and this item therefore, only refers to a hypothetical situation for which there is no teaching in Lea and detection under this situation is ineffective and of no practical use. Furthermore, repetitive process of stopping and starting fluid flow in flow cytometry would invariably result in mixing up of the labeled particles, hence, contravening the principle of keeping large separation between the particles, as required by hydrodynamic techniques such as that of Lea.

Magnification

The method of the invention is carried out at a low magnification (smaller than 20:1), whereby it is possible to detect all particles (plurality of particles) in a large volume in one or a few exposures. The low magnification offer several advantages, such as increased area of observation and increased depth of focusing, implying increased volume exposed to the detection device (Page 23, lines 3-34). Although, Lea mentions that the area of the image of each particle at the said array is approximately the same as the area of at least a single charge coupled device, indicating a 1:1 relation but the cited art fails to recognize the magnification as a result-effective-variable that would achieve the recognized result of simultaneously detecting signals from a

plurality of weakly labelled particles. Therefore, indication of magnification 1:1 relation *per se* in Lea would not suggest to a person skilled in the art to modify the magnification to less than 20:1. (*In re Antonie*, 559 F.2d 618, 195 USPQ 6 (CCPA 1977))

Blood cells

The Office Action co-relates blood cells of Lea et al. with preferred form of particles, i.e. blood cells of the invention, and concludes that Lea's reference teaches assessing particles having less than 1×10^6 detectable positions. However, there is neither any reference in the cited art that the detectable positions in the blood cell are less than 1×10^6 nor any teaching for such low number of detectable positions. Furthermore, it is to be noted that DNA (cell core) binding produces a much high number of (approximately 3×10^9) detectable positions for the same blood cell as opposed to that obtained from surface binding, which will produce less than 1×10^6 detectable positions. Therefore, use of the same cell is certainly not an indication of available active detectable positions. Lea's hydrodynamic method will work well with blood cells having DNA (cell core) binding because this produces detectable signal under particle flow condition because of a large number of detectable positions. However, such flow assessment method will not work reliably with blood cells having surface binding reagents.

Superparamagnetic beads

The Office Action further points out that the particles are bound to reagents in the form of superparamagnetic beads or via a sandwich complex (col. 4, line 25 - col. 5, line 5). However, the cited art further mentions that the magnetic particles are typically attached to selected cells, which after processing, may be more convenient to lyse and only the nuclei which have previously been stained by a fluorescent dye are counted (col. 4, lines 59-62). The applicant submits that the usage of the word "may" in the previous statement demonstrates possibility of

higher convenience in lysing a cell after the processing and not whether the cells are lysed. In other words, in Lea's method, the cells are necessarily lysed. This clearly indicates that before the use of the hydrodynamic technique, the Lea method requires breaking the cellular membrane and creating a lysate, followed by counting only the stained nuclei (cell core), thereby indicating production of high number of detectable positions, as described above, and hence making the cited art principally different from the present invention.

Conclusion: Lea et al.

There is no evidence or suggestion in Lea reference relating to the technical problem that is solved by the invention, thereby lacking enough teaching to suggest a skilled person to relate Lea reference with proposed solution of the present invention. Consequently, from Lea's teachings, a skilled person is restricted only to a hydrodynamic technique (flow cytometry) and with particles having a high number of detectable positions. In fact, the fundamental difference, among others, between the Lea and present invention is the technical problem that a skilled person will face. Because, Lea's work operates in a hydrodynamic area and does not touch upon the issue of particles having low amount of visual label, there is no probability that a skilled person will look and interpret the teachings of Lea to solve the technical problem.

Even if a person skilled in the art with application of common sense attempts to employ the teachings of Lea, he will have to think and work in lines of standstill (hydrostatic) positioning of the sample to solve the technical problem. This is a non-obvious and conceptually divergent technique from Lea's hydrodynamic technique. There is also no hint provided to consider using the method of Lea in the special case of assessing qualitative and other quantitative parameters of particles, on which labelling agents bind to analytes present only in a low number on each particle.

In view of above assessment, the applicant believes that currently amended claim 87 and all claims dependent thereon are non-obvious in view of Lea et al. and its combination with any other prior art document because Lea et al. is not a relevant art for combination with any other art since it is teaches away from the principle employed in the present invention.

<u>The invention is non-obvious over combination of Lea et al. (US 5,428,451) and Kaplan et al. (US 6,280,961)</u>

Scope & Content of Kaplan

Kaplan discloses a system for analyzing cellular materials wherein the density of analytical sites on the surface of the cell is low, and in this regard less that 1 X 10^6 per particle. Kaplan mentions that hard to detect analytes may be readily detected by the increase in the labelling of the cell or particle caused by the amplification of the labelling molecule (col. 2, lines 17-21). This is further exemplified at col. 7, lines 12-43 where cells or particles are tyramide coated for physical separation using serial amplification. Therefore, Kaplan is not relevant to the teachings of the invention because of the low density of particles; an analyte dependent enzyme activation system (ADEAS) must be used to provide an enhanced signal. As such, Kaplan teaches away from the principles of the present invention insofar it suggests to one of the skilled in the art that the chemical ADEAS amplification system must be employed in analyses of this type. (See, *In re Haruna*, 249 F.3d 1327, 58 USPQ2d 1517 (Fed. Cir. 2001).)

Labelling Agent & catalytic activity

In the present invention, the labelling agent is a compound that is capable of emitting, absorbing, attenuating or scattering electromagnetic radiation to result in the generation of a detectable electromagnetic signal and the labelling agent selectively and directly binds to an analyte position. However, in Kaplan, detectable markers are added after tyramide coating to

facilitate physical separation. Tyramide coating over the surface of cells or particles involves catalytic activity of the enzymatic portion of a binding partner and an appropriate substrate resulting in inducing chemical changes in other substances. To be able to detect and physically separate labelled particles, Kaplan utilizes a method for tyramide coating cells or particles using serial amplification, therefore, principle of labelling of particles/cells in Kaplan is different from that in the present invention and incorporating teachings of Kaplan in that of Lea makes it different from claims of the present invention.

Standstill condition

The Office Action states that Kaplan's use of plates and dishes suggests using standstill sample. However, the reasons included under following sections of Hansen et al. are applicable to the combined teachings of Lea and Kaplan, where it is emphasized that any attempt to incorporate standstill sample in the hydrodynamic flow cytometry mechanism of Lea is in violation to the principle of operation of Lea's method. Therefore, any conclusion suggesting such a combination is unreasonable.

Labelling

Difference in working principles of Kaplan and Lea is further illustrated in Kaplan through its various examples where washing steps are used. The washing of the plate indicates that the labelled particles are supported on a solid support, and are therefore, immobilized. This allows the particles to withstand the washing. However, such washing steps are incompatible with the hydrodynamic method of Lea because the particles are not immobilized on any solid support. In fact, the sample particles in Lea are in constant continuous movement through the optical cell. Therefore, incorporating teachings of Kaplan in Lea makes Lea's method unworkable.

Conclusion: Kaplan et al.

The working principle of Kaplan is different from that of the present invention because the cited art makes the use of amplification of the labelling molecule as a mandatory requirement. Therefore, Kaplan is inconsequential to determine inventiveness of the present invention because it fails to correctly disclose at least the labelling mechanism of the present invention. Therefore, combining Kaplan's teaching to that of Lea's would result in a combined system, which is principally, different from the one claimed in the present invention.

Currently amended claim 87 and its related dependent claims are therefore non-obvious over the combined teachings of Lea et al. and Kaplan et al.

The invention is non-obvious over combination of Lea et al. (US 5,428,451), Kaplan et al. (US 6,280,961) and Hansen et al. (EP1180675)

Scope & Content

Hansen et al. discloses a method and system for assessment of properties of particles in a liquid sample. The scope and content of Hansen et al. relates to assessing by staining the particles with stains which are known to stain DNA monomers (about 3 x 10^9 available detectable positions). Accordingly, Hansen et al. discloses methods for assessment based on staining abundant molecules in the cells. Under such conditions, the signal accumulated from each cell is relatively high and the signal to noise ratio is correspondingly low so that distinction between signal from particles and background is facilitated. Although, Hansen et al. teaches assessment of particles under stand still positions (Para 70), but there is no teaching in Hansen et al. to solve the technical problem of "How to measure labelled particles with a low amount of a visual label" less than 1 x 10^6.

Hansen combined with Lea & Kaplan

In combination with Lea et al., it is observed that Lea et al. teaches assessment of one particle at a time by employing hydrodynamic flow cytometry technique, Kaplan et al. relies on having low density of analytical sites on the surface of the cell but with ADEAS amplification on a plate/dish whereas Hansen et al. primarily assesses stand still particles by staining abundant molecules in the cells. It should be observed that the cited arts are contradictory in nature, if combined, because bringing the speed of a flow cytometer to zero is not in compliance with the working principle of flow cytometry and in stand still particle position, Lea's teaching would seize to operate in the area of flow cytometry. The only common feature between Lea and Hansen prior arts is their incapability to assess particles having low number of detectable positions. If the modification or combination of the prior arts, as proposed in the Office Action is applied, then the principle of operation of the prior art invention being modified would change, therefore, the teachings of the references are not sufficient to render the claims *prima facie* obvious. (*In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959)).

Conclusion: Hansen et al.

The applicant concludes that an artisan having common sense at the time of the invention would not have reasonably considered embedding a hydrostatic teaching of Hansen et al. within an existing hydrodynamic teaching of Lea et al. in the manner suggested by the Examiner.

Amended claim 87 and all claims dependent thereon are hence non-obvious in view of Lea et al. combined with Kaplan et al. and Hansen et al.

Other Combinations: Sub-Claims

Although, other cited arts are not taken as a basis to contest obviousness of the main claim. However, the arguments presented against the other citations are described to illustrate

why they are either not combinable with the teachings of the primary citation Lea et al. or why the citations are not relevant to the present invention. As such, the skilled person would not identify these citations and their teachings to conceive the sub-claims of the present invention. Further, as identified under individual sections below, most of the following combinations rely on detecting particles having analyte detectable positions more than 1×10^6 per particle. The specific rejections for each of the following combinations are also addressed in this submission.

In addition to specific combination based rejections, the Office Action also relies on Hansen's teaching of binding a fluorochrome to DNA within a somatic cell and Kaplan's teaching that the analytes are present at less than 20,000 molecules/cell (or particles). The applicant submits that, because Hansen teaches staining DNA monomers, therefore, as described earlier, it discloses methods for assessment based on staining abundant molecules (about 3×10^9 available detectable positions) in the cells. As regards Kaplan, it has already been established previously that although Kaplan discloses a low copy number but ADEAS must be used, in Kaplan, to provide an enhanced signal. Consequently, both Hansen and Kaplan are different from the teachings of the present invention.

The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Singer et al. (US 5,728,527)

In Singer et al., a method of nucleic acid hybridization in living cells is described, which is useful for detecting a specific nucleic acid in a cell or tissue, for selecting cells based on the expression or presence of a specific nucleic acid, and for monitoring the amount and location of a specific nucleic acid under various inducing or inhibiting conditions. The detection of target nucleic acid is based on incubating the living cell or tissue with a labelled single-stranded oligonucleotide probe, thereby making it a cell core labelling mechanism rather than a surface

labelling mechanism. As described under Hansen et al. argument, DNA staining results in abundant labelled molecules (about 3 x 10^9 available detectable positions) in the cells. Under such conditions, the signal accumulated from labelled oligonucleotides of each cell is relatively high and the signal to noise ratio is correspondingly low so that distinction between signal from particles and background is facilitated. This method of oligonucleotide binding will not work if the active detectable positions are as low as $1X10^6$ or lower, as the case in the present invention.

In addition, Singer et al.'s method, the assessment of the species is based on taking photomicrographs (col. 10, lines 22-29) of stained cells fixated on e.g. a glass slide (col. 9, line 65 – col. 10, line 3 and in col. 12, line 60 – col. 13, line 2). The cells that are to be analysed are actually grown on glass cover slips (col. 11, lines 33-39). Lea et al. on the other hand relates to a method for the detection of particles in a liquid sample and hence the detection method of Lea et al. and Singer et al. cannot be combined. Therefore, a skilled person would not be prompted to combine the teachings of Lea et al. and Singer et al. as these two methods relates to un-combinable detection methods.

The mere fact that Singer et al. describes that it is possible to determine the content of particles fixated on a glass slide does not provide the skilled person with any expectation of success with respect the possibility of assessing particles in a liquid sample based on labelled analyte having less that 1 x 10^6 position in a completely different system.

The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

In situ hybridization, according to this art clearly indicates that the binding to specific sequence takes place exactly in place where it occurs, that is, chromosomal DNA, which is inside the nucleus. This makes the cited art method as a cell core binding and leads to same conclusion that the detectable positions generated would be much higher than that in the present invention. Similarly, the cited art mentions that the target complements are nucleotide, which indicates use of cell core binding while detecting two colour labels. In combination with the teachings of Hansen and Kaplan, the resulting labelling would still be a core labelling and use an amplification system to enhance the signals. However, label detection in the present invention is based on surface binding particles.

Claims 93, 103, 110-111 and 114, as applied to claims 87-88 and 92 are hence non-obvious in view of Lea, Kaplan and Hansen combined with Singer et al.

<u>The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Connors et al. (US 5,726,009)</u>

Connors et al. relates to a method of using an in vitro culture system to measure the cell proliferation and cell viability of human tissues. Connors et al. measures number of cells in a histocultured tissue sample by incorporating a DNA-synthesis marker into the proliferating cells. That is, the number of proliferating cells in the tissue is indicated by the number of cells in the histocultured tissue sample having metabolically incorporated the DNA-synthesis marker into the cellular DNA during cell division associated with cell proliferation. This art relies on DNA binding, which invariably provides a high number of detectable positions, as described under Hansen et al. section. In combination with Lea's method, Connor's teaching still lack the information or hint addressing the technical problem of assessing particles with low number of detectable positions. Also, Connors et al. detect histocultured sampled under light microscopy,

which is contradictory to the working principle of flow cytometry, which relies on assessing particles in a flow condition one at a time.

The combination lacks any clue about assessment of particles having low number of detectable positions and contrary working principles of arts for assessment of particles make them incompatible and an improper combination. Further at col. 3, lines 45-67, Connors disclose that the three dimensional structure of the tissue sample is maintained during culturing and subsequent optical characterization. The method of Connors et al. is hence fundamentally different from the method according to Lea wherein a liquid sample flows by the detection elements.

In relation to the magnification, Connors et al. describes in col. 7, lines 35-37 that a magnification of 200:1 is used and there is no disclosure in Connors wherein a magnification of below 20:1 is used. Although, the Office Action states that "<u>may be</u>" magnified encompasses "<u>not magnified</u>" as well. However, the applicant submits that Connors states that the magnification is <u>typically</u> around 200:1 and not may be magnified. Therefore, the applicant respectfully submits that the magnification teaching of Connors is incorrectly interpreted.

The analyte detection on the surface of an organelle is incompatible with the teachings of Lea et al. because Lea et al. relies on cell core binding. If this teaching in somehow incorporated in the method of Lea et al., the detectable signal produced would be low in strength. Therefore, no skilled person would implement this in the flow cytometry method of Lea et al. because it would lead to unreliable results. The additional teachings of Hansen and Kaplan do not provide any additional teaching to overcome this limitation.

Claim 94, when read with claim 87, is hence non-obvious in view of Lea, Kaplan and Hansen combined with Connors et al.

The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Riabowol (US 5,877,161)

Riabowol relates to cyclin D1 as a regulator of cell proliferation. The scope of Riabowol is to regulate cell growth using cyclin D1 rather than the counting method *per se*. Riabowol, therefore, utilizes commonly available techniques, namely autoradiography or fluorography to identify the growth. The labels used in Riabowol's method include both radioactive and fluorescent dyes. It is to be noted that the dyes are incorporated into a compound a substance that is readily detected. Therefore, it is clear from "incorporated into a compound", that Riabowol utilizes cell core labelling as opposed to surface labelling. Cell core labelling provides a large number of visual labels, thereby making common fluorography technique workable. As the intention of Riabowol was not to address the technical problem of assessing parameters of particles with low visual labels, there is no teaching that can be found in Riabowol addressing such a problem.

Also, when the cells are studied, they are fixated on a slide (col. 6, lines 43-48).

In absence of any specific assessment technique provided in the Riabowol citation, it is evident that there is no possibility of supplementing the shortcomings of Lea et al.'s with that of Riabowol's. Therefore, no skilled person would ever be motivated to combine the teachings, leave alone with reasonable expectation, of the Lea et al. and Riabowol because even in combination the cited arts do not hint using a hydrostatic method for assessing particles with low visual labels.

The applicant submits that although Riabowol mentions cyclin D1, however as mentioned above, the method of Riabowol is based on cell core labelling (label incorporated into a compound). Therefore, detection of a state of quiescence, hyerplasticity, or neoplasia in a biological sample is made possible owing to large number of detectable positions generated. Riabowol does not teach surface binding and in combination with Lea et al., it would still be a process addressing cell core binding and therefore would not provide any teaching on how to successfully use cyclin D1 for low visual labels.

Claims 102, 104, 107, and 131-132, when read with claim 87 are hence non-obvious in view of Lea, Kaplan, Hansen combined with Riabowol.

<u>The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Draetta et al. (US 5,691,147)</u>

Draetta et al. relates to novel proteins of mammalian origin which can associate with the human cyclin dependent kinase 4 (CDK4). The art briefly mentions a probe/primer, comprising a label group attached to a tissue and being able to detect the label groups, which are selected from radioisotopes, fluorescent compounds, enzymes, and enzyme co-factors. Draetta mentions that detection of presence of absence of the genetic lesion is performed by in situ hybridization of the probe/primer to the nucleic acid. The attachment of probe/ primer to the nucleic acid is a form of core binding. This results in a high number of detectable positions, making assessment of the particles, even under flow condition, possible.

There is no teaching relating to a specific optical detection method for the species and also detection of low number of detectable positions. There is no additional information, which could be combined with Lea et al.'s work for assessing parameters of particles having low detectable positions. Furthermore, even if a combination is tried, the derived method would work

only with particles having high number of detectable positions and would still be in the area of flow cytometry because Lea's work only suggests flow cytometry technique. Therefore, the combination of works of Draetta et al.'s and Lea et al.'s does not provide any hints to making an assessment of particles with low detectable positions at standstill condition, as claimed in the method disclosed in the claims of the present invention.

In situ hybridization, according to this art clearly indicates that the labeling of the probe/primer to the nucleic acid takes place exactly in place where it occurs, that is, inside the nucleus because nucleic acid is part of the nucleus. This makes the cited art method as a cell core labeling and leads to same conclusion that the detectable positions generated would be much higher than that in the present invention. Therefore, detection of CDK4 in the cited art is possible because of higher number of detectable positions. There is no indication in the art if Draetta et al.'s method would produce a reliable result for cell surface labeling of CDK4. Hence, Draetta et al. does not supplement the shortcomings of the base citation of Lea et al.

Claims 105, 107, and 135, when read with claim 87 are hence non-obvious in view of Lea, Kaplan, Hansen when combined with Draetta et al.

The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Bitler et al. (US 6,379,882)

Bitler et al. describes a method for selecting therapeutic agents for in vivo treatment. A number of different methods for detecting fluorescently stained cells are briefly mentioned: flow-cytometry (col. 12, line 48), microscopy (e.g. col. 13, lines 3-4; col. 13, lines 9-12; and col. 14, line 5) and so forth. The flow cytometry technique of Bitler does not provide any additional information over Lea et al. to a skilled person to devise the present invention because they are still in the same area of flow cytometry. This, therefore, would have the certain limitation of not

being able to assess standstill particles having a low number of detectable positions. On the other hand, microscope based method is in contravention with the working principle of flow cytometry method of Lea et al. because, under no circumstances, a skilled person would relate assessment of a sample on a slide with a sample that is under flowing condition, where one cell is analyzed at a time. Furthermore, Bitler et al. provides no hints to adapting the method according to Lea et al. to having the liquid sample at standstill during the optical detection.

The applicant submits that Bitler et al. utilizes Terminal Deoxynucleotidyl Transferase Mediated dUTP Nick End Labeling (TUNEL). TUNEL depends on labelling the DNA, making Bitler a cell core labelling as opposed to the surface labelling. Therefore, detection of phosphatidylserines targeted with Annexin V for particles with low visual labels is not demonstrated in the Bitler et al. and therefore, does not provide any additional information for combination with the cited art of Lea et al.

Claims 106, 107, and 136, when read with claim 87 and all claims dependent thereon are hence non-obvious in view of Lea, Kaplan, Hansen when combined with Bitler et al.

The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Mathies et al. (US 6,100,535)

Mathies et al. describes a system for detecting electrophoretic separations in capillary tubes. When using a system for electrophoretic separation to analyse samples containing variously-sized particles (such as stained DNA fragments), the particles are spatially separated by an electrical field applied over the sample. When subjecting a sample containing a number of differently-sized DNA fragments stained with a fluorescent dye to an electrophoretic separation, the DNA fragments of same size will form a coherent population at a location in the capillary tube determined by the fragment size. Fluorescence emitted from the stain molecules when

exposing the capillary tubes to an excitation light source is seen as bands. The content of the examined sample is identified by comparing the position of the bands of the sample under analysis with the location of the bands from stained DNA fragments in a reference sample. In the method according to Lea et al., electromagnetic signals from the individual particles are identified as distinct from the background and the liquid sample flows during the optical inspection. The system according to Mathies et al. hence relates to a measurement technique that is incompatible with the method according to Lea et al. and the person skilled in the art would not be prompted by Mathies to adapt the system according to Lea et al. to be similar to the system according to the present invention.

Furthermore, Mathies mentions that light from the sample volume, for example fluorescing DNA fragments, is collected by the objective. Use of DNA fragments is a clear indication of cell core labelling, which results in a large number of detectable positions. Therefore, Mathies also fails in providing any hints regarding assessment of particles having low number of detectable positions.

The mere fact that Mathies et al. describes that it is possible to separate DNA fragments in a sample and detect populations of these fragments from their locations, does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles based on labelled analytes having less than 1×10^6 positions.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Mathies et al. without the use of impermissible hindsight.

The present application mentions in paragraph 173 that confocal scanning optical microscopes are known in the art and offer a number of advantages over traditional optical

microscopes. One main advantage of a confocal scanning microscope is that it provides optical sectioning of a sample because it attenuates light which is not in focus. Thus, only light which is in focus contributes to the final image. The use of confocal scanner in the present application is in conjunction with claim 87, which the applicant submits is non-obvious over the combination. Therefore, limitation of confocal scanner in the present invention is therefore non-obvious over Mathies.

Claim 119, when read with claim 87 is hence non-obvious in view of Lea, Kaplan, Hansen when combined with Mathies et al.

The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Sherley et al. (US 5,741,646)

Sherley et al. relates to a method for determining the effect of a substance on cell growth kinetics. Sherley et al. gives a general reference to the use of bromodeoxyuridine-Hoechst dye fluorescence quench procedure to visualize and quantify the daughter cell products of induced stem cell division. It is to be observed here that Sherley et al. does not give information about any particular assessment technique except for the use of a fluorescent dye, and Bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) dye is commonly used in the detection of proliferating cells in living tissues. BrdU is usually incorporated into the newly synthesized DNA of replicating cells, thereby, making it a core binding and leading to high number of detectable positions, as already mentioned before.

Because of absence of teaching with respect to assessment technique and use of a core binding technique employed in Sherley et al., leading to a higher number of detectable positions, it is evident that limitations of Lea et al. are not supplemented by the teachings of Sherley et al. Therefore, a combination of Lea et al. with Sherley et al. would still be in flow cytometry

domain. Consequently, it is highly unlikely that a skilled person would try to look for a standstill solution approach for particles with low number of visual labels in Sherley's art.

The applicant asserts that although Sherley et al. refers to detection of p53 but in such detection a murine cell line is stably transformed with an exogenous DNA molecule. The use of exogenous DNA, as mentioned above, clearly indicates use of a cell core binding as opposed to surface biding. Therefore, use of p53 alone is not an indication of workability of p53 with Lea in the same way as in the present invention.

Claim 102, 104-105, 107, 131, and 133, when read with claim 87 are hence non-obvious in view of Lea, Kaplan, Hansen when combined with Sherley et al.

The invention is non-obvious over combination of Lea et al., Kaplan et al., Hansen et al. and Harvey et al. (US 5,344,760)

Harvey et al. relates to a diagnostic/prognostic method for squamous cell carcinoma or urinary tract cancer comprising detecting and/or quantitating in a mammalian urine or bladder wash sample a portion of the epidermal growth factor (EGFr) which comprises substantially the EGFr ectodomain and which has a molecular weight in the range of from about 90 kilodaltons (kd) to about 115 kd, wherein an elevated concentration of said portion above the concentration for healthy mammals or for those with benign disease indicates the presence of squamous cell carcinoma or urinary tract cancer. Harvey does not provide any specific information to address the technical problem of "how to measure particles with low visual labels". More specifically, there is no information regarding the number of detectable positions and therefore, does not provide enough teaching to a person skilled in the art to relate Harvey et al.'s method to combine it with Lea et al.'s.

The applicant submits that although Harvey et al. teaches detection of EGFR but in absence of any information to solve the technical problem, it would be incomprehensible for a skilled person to correlate teachings of Harvey et al. with that of Lea et al. Therefore, no skilled person looking for a solution regarding detection of particles with low visual labels would combine the cited art of Harvey et al. with Lea et al. with an expectation of success.

Claims 102, 104-105, 107 and 134, when read with claim 87 are hence non-obvious in view of Lea, Kaplan, Hansen when combined with Harvey et al.

Summary

The applicant has precisely and persuasively showed that the base citation of Lea et al.'s is inapplicable and limited because:

a) Lea et al.'s teaching is incapable of providing any hint to address the technical problem of "How to assess parameters of particles having low visual labels";

b) More importantly, the scope of Lea et al. does not cover or provides any hint for assessment of particles, which are at standstill condition because hydrodynamic flow cytometry technique of Lea et al. is in contradiction with the working principle of hydrostatic operation of the present invention. Although, flow control is suggested in Lea et al.'s work but such control is limited so long as the control complies with the working principle of flow cytometry, that is, the sample is under flow condition and the particles are assessed sequentially one at a time.

Combinations of Lea et al. with Kaplan and Hansen is inappropriate because:

a) Hansen et al. teaches DNA binding, which eventually leads to a high number of visual labels, thereby deviating from the scope of the present invention; and

b) Standstill assessment of Hansen et al.'s work is incompatible with that of Lea et al.'s because the teachings of these citations have different working principles. Under no

circumstance, a standstill assessment is possible within the functioning of a flow cytometer. Therefore, the combination of work of Lea et al. and Hansen et al. is an improper one, leading to uncertainty in the mind of skilled worker.

c) Kaplan teaches use of ADEAS amplification to allow detection of particles where the density of analytical sites on the surface of the cell is low. In absence of the amplification, hard to detect analytes may not be readily and reliably detected. Use of such amplification is against the working principle of the present invention.

Combinations of Lea et al. with other cited works have at least one of the following drawbacks:

a) Assesses particles with a high number of visual labels;

b) Are restricted to the area of hydrodynamic flow cytometry techniques;

c) Are adapted to work in an experimental condition, for example light microscopy (Connors et al.), which makes the combination with Lea et al.'s flow cytometry technique unsuitable.

In view of different scope of the base citation (Lea et al.) from that of the claimed invention, no hint in any cited art regarding assessment of particles having low visual labels, unworkable combinations because of different scope (working principles) of the cited methods when combined with Lea et al.; the applicant respectfully, yet firmly, traverses obviousness rejections of the Examiner and respectfully states that:

a) the rejections of the Examiner are primarily caused by hindsight bias and the arguments in the Office Action are reliant upon ex post reasoning.

b) In the instant case, the applicant concludes that a person of ordinary skill in the art having common sense at the time of the invention would not have reasonably looked to Lea et al. to solve the technical problem, which is solved by the present invention.

c) Therefore, the applicant concludes that an artisan having common sense at the time of the invention would not have reasonably considered embedding a hydrostatic assessment technique within an existing hydrodynamic assessment technique.

d) Further, using an amplification mechanism, which is altogether a different working principle does not relate to the solution claimed in the present application.

The Affidavit

Applicant presents herewith the affidavit of Professor Rolf Henrik Berg addressing issues of obviousness raised by the Examiner in the subject Office Action. As detailed in the affidavit, Professor Berg has expert knowledge in the relevant technical field. In his affidavit, Professor Berg specifically discusses the Lea, Hanson and Kaplan prior art and advances his expert opinion as to, *inter alia*, why the Lea system does not and cannot operate under standstill conditions and why it must operate on single particles which have a high number of detectable positions. Professor Berg addresses the Hanson prior art and presents his opinion as to why the Hanson system must operate on particles having a high number of detectable positions. Professor Berg further advances his opinion on the Kaplan prior art and explains why the teaching of Kaplan is of the need to use a very specialized chemical amplification system (ADEAS) when analyzing cellular material having a low number of reactive sites. Professor Berg further explains as to why it would not be obvious, or even possible, for one of skill in the art to combine the teachings of Lea, Hanson and Kaplan as suggested by the Examiner so as to approximate the present invention.

The arguments presented in this affidavit are very relevant to the issue of patentability and must be taken into consideration. The Court of Appeals for the Federal Circuit in the case of *In re Sullivan et al.*, slip op. 2006-1507 S.N. 08/405,454 (CAFC 2008), held that the Board of Appeals committed error when it failed to give consideration on the record to evidence submitted in the form of expert affidavits with regard to the issue of obviousness of an invention in view of prior art. The Manual of Patent Examining Procedure likewise requires that timely presented objective evidence in the form of affidavits or declarations directed to the issue of nonobviousness must be given consideration by the Examiner (MPEP 716.01(a)). Accordingly, the Examiner must consider and address this affidavit evidence on the record.

Conclusion

In view of the amendments, remarks, and affidavit submitted herewith, Applicant respectfully submits that all objections and rejections are overcome. The application is in condition for allowance. Any questions, comments, or suggestions which the Examiner may have which will place the application in still better condition for allowance should be directed to the undersigned attorney.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 07-1180.

Dated:

Respectfully submitted,

By

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